Partial Fractionation of Water-Soluble Nitrogen from Coastal Bermuda Grass Growing under Various Soil Nitrogen Levels

E. R. IBERT, F. L. FISHER, and J. F. FUDGE

Texas Agricultural Experiment Station, College Station, Tex.

Fresh samples of Coastal Bermuda grass, maintained under different nitrogen levels (0, 13, 75, 125, and 225 pounds of nitrogen per acre every 4 weeks), were collected twice a week through two nitrogen cycles during the 1955 growing season. Freshly harvested plant material from the selected nitrogen levels was clipped into approximate 1-inch lengths and individually mixed. A portion was dried for moisture content, then analyzed for total nitrogen. Portions of the fresh plant material were put in a high speed blender with 400 ml. of distilled water for 10 minutes. An aliquot of this extract was used for determining water-soluble NH_4^+, NH_2^-, NO_2^- + NO_3^- content of this fresh material. Each of these nitrogen fractions showed a positive correlation with the level of total nitrogen in the plant and the level of soil-solution nitrogen.

NITRATE TOXICITY IN ANIMALS (*1*, *6*) has been the frequent result of the use of high rates of nitrogen fertilizer in the production of hay. A study, growing Coastal Bermuda grass under very high levels of nitrogen fertility, was started in 1953, to measure quality and quantity of hay produced. Quantity was to be measured by clipping small plots, while quality was to be measured by chemical analysis and by feeding trials using rabbits. The management of the rabbits presented such a problem that this phase of the study was dropped without securing any data of value. In 1955 a partial fractionation of the water-soluble nitrogen was used instead of the feeding trials as a means of evaluating the quality of hay produced.

Field Practices

This experiment with Coastal Bermuda grass (*4*, *5*) under varying nitrogen levels with supplemental sprinkler irrigation was started near College Station, Tex., in June 1953. The experimental design was a randomized block with three replications. Treatments consisted of split applications of different rates of nitrogen as shown in Table I. The Lufkin fine, sandy loam soil selected is typical of large areas of East Texas and the southern part of the United States. It has a shallow sandy surface with a slowly permeable subsoil. The soil was medium in organic matter (2.0%), very low in phosphorus (11 p.p.m.), and low in available potash (120 p.p.m.), and had a pH of 5.4.

The entire area was limed with calcium carbonate to a pH of 7.0 just prior to sprigging of the Coastal Bermuda grass. The lime and a broadcast fertilizer treatment of 30-100-100 [30 pounds of nitrogen (N), 100 pounds of available phosphoric acid (P_2O_5), and 100 pounds of potassium oxide (K_2O) per acre] was worked into the upper 3 to 4 inches of soil. Sprigs of Coastal Bermuda grass were set on 24-inch centers on June 10. The area was mowed at a height of about 3 inches and the growth removed in mid-September. A uniform application of nitrogen, 30 pounds per acre, was applied and watered in with about 2 inches of water. The entire area was top-dressed with a 0-100-100–fertilizer treatment in February 1954 and differential nitrogen treatments were initiated. Immediately following any fertilizer application, at least 1 inch of water was applied by sprinkler. Soil moisture was maintained at a high level throughout the experiment. During July and August, when temperatures daily approached or passed 100° F., irrigation water was applied at the rate of about 2 inches a week.

Table I. Rate of Application of Nitrogen in Pounds per Acre

1954[a]		1955[b]	
Per application	*Per season*	*Per application*	*Per season*
0	0	0	0
20	100	13	75
40	200	25	150
80	400	50	300
120	600	75	450
160	800	100	600
		125	750
		138	825
		150	900
		175	1050
		200	1200
		225	1350

[a] Five applications of nitrogen as ammonium sulfate on Feb. 22, April 28, May 20, June 21, and Aug. 9. Plots harvested on April 12, May 17, June 14, July 15, Aug. 12, Sept. 9, and Oct. 7.

[b] Six applications of nitrogen as ammonium nitrate on March 10, May 13, June 16, July 14, Aug. 12, and Sept. 9. Plots harvested on May 13, June 13, July 13, Aug. 10, Sept. 7, and Nov. 3.

Nitrogen deficiency was observed on all plots in September 1954. To correct this nitrogen deficiency, 250 pounds per acre of nitrogen, as ammonium sulfate, was applied on half of each plot. An immediate increase in vigor and growth was noted following this treatment. Subsequently (November 1954), soil samples were taken from all plots. Chemical analyses of these samples indicated that the nutrient balance was not being maintained, particularly where high quantities of nitrogen had been applied (Table II). Therefore during 1955, 1 pound of phosphoric acid and 2 pounds of potassium oxide were applied for each 10 pounds of nitrogen. Dolomitic limestone (8% magnesium) was applied at a rate of 30 pounds to 10 of nitrogen. The area was clipped in February and all harvested material removed. Differential nitrogen rates were started on March 10, 1955. During 1955, analyses of soil samples indicated that adequate amounts of the other nutrients had been maintained (Table II).

Typical data on soil solution nitrate (NO_3^-) *vs.* time in days after the application of nitrogen to the soil are shown in Figure 1. Fifty pounds or less of nitrogen per acre per application produced no apparent increase in nitrate ion in the soil solution. The application of higher rates of nitrogen produced an immediate increase in the soil solution nitrate which dropped to less than half the maximum within 16 days, and to the level of the control plots within 30 days. No horizontal diffusion losses were indicated and the nitrate gradient with depth was sharp and uniform. Forage analyses

Table II. Effects of Fertilizer Treatments and Coastal Bermuda Grass on Level of Soil Nutrients, as Shown by Analyses of Soil at End of Growing Seasons, 1954 and 1955

N in Lb./Acre/Yr.		pH of Soil		P_2O_5 in Soil, P.P.M.		K_2O in Soil, P.P.M.		Ca in Soil, P.P.M.	
1954	1955	1954	1955	1954	1955	1954	1955	1954	1955
000		7.0		28		173		940	
	75		7.0		46		134		1000
100		6.8		28		139		1120	
	150		6.8		29		132		1000
200		6.4		16		117		970	
	300		6.4		40		124		850
400		6.0		13		94		750	
	450		5.6		43		137		970
600		5.6		8		77		940	
	600		5.4		35		106		940
	750		5.7		44		160		950
800		5.0		10		69		900	
	825		5.2		45		125		930
	900		5.1		46		134		810
	1050		4.8		54		164		950
	1200		4.9		47		145		950
	1350		4.9		48		194		870

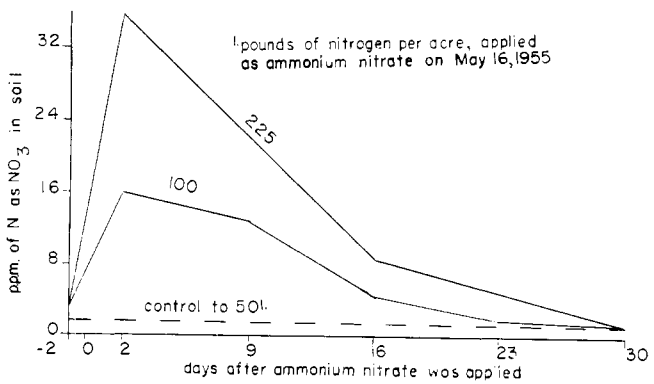

Figure 1. Effect of time on level of soil nitrate under Coastal Bermuda grass, College Station, 1955

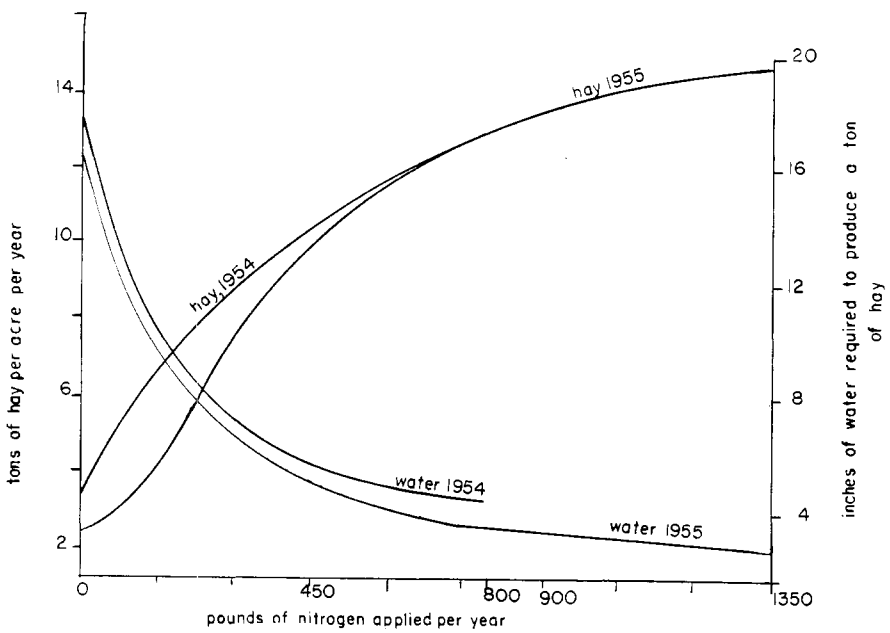

Figure 2. Effect of nitrogen on hay yield and water utilization of Coastal Bermuda grass, College Station, 1954–55

indicate 70% of the applied nitrogen was recovered. Percolation losses of nitrates were thought to be small.

Data on production of hay and the utilization of water in 1954 (Figure 2) were practically the same as in 1955. Increases in yield due to nitrogen fertilization were almost identical for both years, although the 1954 season showed a slightly greater efficiency in the use of applied nitrogen at the lower rates. Yield curves for the 2 years (Figure 2) blend into one curve at about 800 pounds of applied nitrogen and 13 tons of hay. Nitrogen applied in 1955 apparently was sufficient to obtain a leveling off of forage production (Figure 2). No significant differences in hay yield (1955) were evident at rates higher than 175 pounds of nitrogen per acre per application, while this and higher rates were significantly better than lower rates (*4*, *5*). Nitrogen removal of the harvested forage showed a similar pattern. The data for 1954 gave no indication of the maximum removal of nitrogen in the hay (Figure 3), however, the 1955 data indicate that it is somewhat under 700 pounds per acre per year.

Laboratory Practice

High nitrate ion concentration in forage has been shown to be toxic to animals (*1*, *6*). Nitrate toxicity has sometimes been associated with high rates of nitrate fertilization. From the observations that the nitrate was rapidly disappearing from the soil solution (Figure 1), it was decided to determine the nitrate ion concentration in the green forage. A method for the determination of ammonia and nitrate in a single sample (*3*), using heavy magnesium oxide and Devarda's alloy, was employed. The nitrate fraction was erratic unless sodium hydroxide was used to remove the pyrolysible-hydrolysible ammonia. The Devarda reduction was carried out after the removal of this fraction.

The hand-clipped grass samples were taken just after 8:00 A.M. and were immediately returned to the laboratory. They were cut into 1- to 2-inch lengths, mixed, and a 20-gram sample was weighed for the fractionation. Another 20-gram sample was taken for a moisture determination and was then used for total nitrogen by the Kjeldahl method. All data shown are calculated on an oven-dry (105° C.) basis. The sample to be fractionated was placed in a high speed blender with 400 ml. of distilled water for 10 minutes. A 100-ml. aliquot, filtered through cheesecloth, and 150 ml. of distilled water were placed in a distilling flask with about 2 grams of heavy magnesium oxide. A few drops of antifoam were added and the solution was distilled (100 ml. in about 30 minutes) into a measured portion of standard acid and

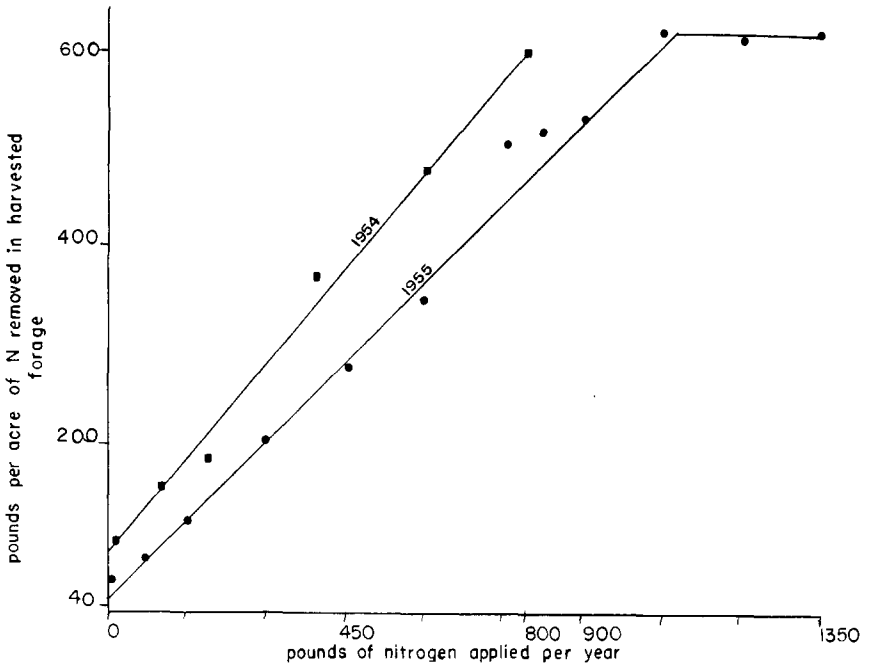

Figure 3. Relation between fertilizer nitrogen and nitrogen removed in Coastal Bermuda grass hay, College Station, 1954–55

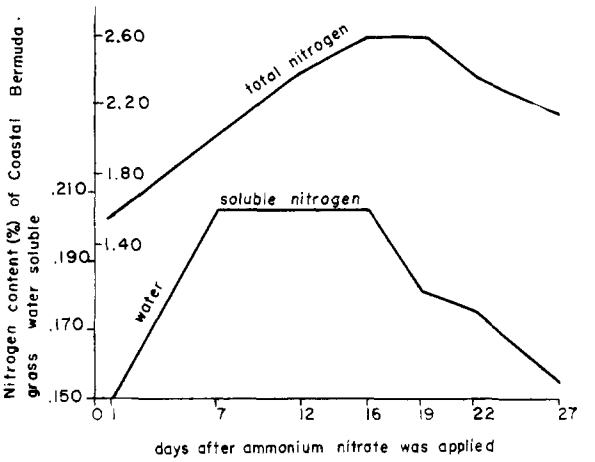

Figure 4. Effect of time on percentage of nitrogen in Coastal Bermuda grass, College Station, 1955

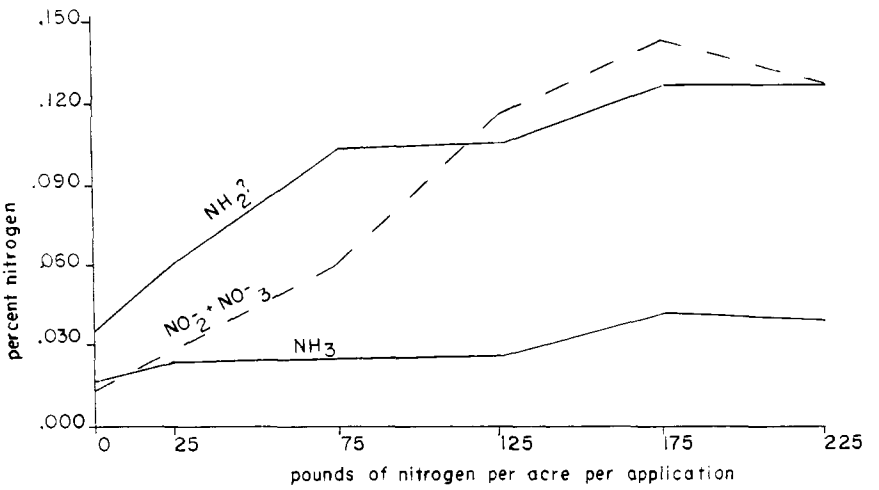

Figure 5. Effect of level of soil nitrogen on water-soluble nitrogen in Coastal Bermuda grass, College Station, 1955

the excess acid was titrated to a methyl red point with a standard base. The amount of acid consumed by the ammonia was calculated to per cent of nitrogen. The residue in the distilling flask was cooled; then 100 ml. of distilled water and 5 ml. of 40% caustic were added and the sample was distilled again. Nitrogen removed in this distillation was reported as NH_2^-? (Hoffman's reaction, pyrolysis, and hydrolysis), calculated to per cent nitrogen. The nitrate-nitrite fraction in the residue was removed by distillation after the further addition of water and 3 to 4 grams of Devarda's alloy. This fraction was calculated to per cent nitrogen.

Results

The total nitrogen (all rates) in the harvested forage *vs.* time in days after the application of nitrogen, is shown in Figure 4. The water-soluble nitrogen content (the sum of the NH_4^+, NH_2^-?, and $NO_2^- + NO_3^-$ as nitrogen) in the forage *vs.* time in days after the application of nitrogen, is also shown in Figure 4. The maximum concentration of nitrogen in the forage was attained within 16 to 19 days after fertilization and then started to decline. The water-soluble nitrogen in the forage on all rates reached a maximum value within a week and held for a week, then declined sharply. The decrease in concentration of all nitrogen components started within 19 days after fertilization, at which time the level of nitrate in the soil solution had dropped to less than 8 p.p.m., even at the highest rates.

A further breakdown of the water-soluble nitrogen (averaged across all sampling dates in one fertilization cycle) components *vs.* the rate of application of nitrogen, is shown in Figure 5. An increase in the rate of nitrogen applied produced a sharper increase in the nitrate-nitrite fraction than in the other fractions. The ammonia fraction was least affected, even though the nitrogen was applied in the ratio of one mole of ammonia to one of nitrate.

The soil nitrate study shows clearly that Coastal Bermuda grass can take in large amounts of nitrogen as either NH_4^+ or NO_3^- in short-time intervals. Under optimum growing conditions, Coastal Bermuda grass is capable of utilizing large amounts of nitrogen without undue stress and of converting this nitrogen into amino acid and proteinaceous material without storing excess NH_4^+ and $NO_2^- + NO_3^-$. The percentage of the total nitrogen content of the plant—i.e., as NH_4^+ and $NO_2^- + NO_3^-$—is almost constant regardless of soil nitrogen level. This suggests that, in Coastal Bermuda grass, high levels of soil nitrogen do not cause more than a temporary accumulation of nitrogen compounds that have been

considered toxic to livestock, which is in close agreement with the work conducted in Georgia (*2*).

Literature Cited

(1) Bradley, W. B., Eppson, H. F., Beath, O. A., *Wyoming Agr. Expt. Sta. Bull.* **241,** 1940.

(2) Burton, G. W., Southwell, B. L., Johnsen, J. C., Jr., *Proc. Assoc. Southern Agr. Workers* **53,** 60 (1956).

(3) Davidson, J., Krasnitz, A., *Ind. Eng. Chem., Anal. Ed.* **6,** 315 (1934).

(4) Fisher, F. L., Caldwell, A. G., *Texas Agr. Expt. Sta., Progr. Rep.* **1731,** 1954.

(5) Fisher, F. L., Caldwell, A. G., Fudge, J. F., *Ibid.*, **1837,** 1956.

(6) Whitehead, E. I., Moxan, A. L., *South Dakota Agr. Expt. Sta. Bull.* **424,** 1952.

Received for review June 18, 1956. Accepted December 28, 1956. Division of Agricultural and Food Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956. Technical Article 2441 from the Texas Agricultural Experiment Station.

METABOLISM OF INSECTICIDES

Metabolism and Excretion of Phosphorus-32–Labeled Diazinon in a Cow

WILLIAM E. ROBBINS, THEODORE L. HOPKINS, and GAINES W. EDDY

Entomology Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Corvallis, Ore.

Phosphorus-32–labeled Diazinon, administered orally to a cow at 20 mg. per kg., is rapidly metabolized and excreted. Only low levels of unchanged toxicant were found in blood and milk samples. About 74% of the dose, excreted as polar degradation products, was accounted for in the urine 36 hours after treatment.

DIAZINON, the organic phosphorus compound, *O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate, has been evaluated against a wide variety of insects affecting plants and domestic animals (*5*). It is an effective toxicant in baits (*4*) and, by residual application, in the control of houseflies in dairy barns (*7*).

McGregor and coworkers (*11*) have demonstrated that Diazinon is a promising chemotherapeutic agent when administered orally or subcutaneously to cattle parasitized by cattle grubs, *Hypoderma lineatum* (De Vill.). It is also effective when applied externally to cattle as a back treatment (*16*).

More recently the distribution and excretion of Diazinon in guinea pigs (*9*) and its penetration and excretion by the American cockroach (*1*) have been studied with the aid of phosphorus-32–labeled Diazinon and paper chromatographic analysis.

To gain a better understanding of its action as a mammalian systemic insecticide and to investigate the nature of its metabolic breakdown products in the bovine body, phosphorus-32 Diazinon was administered orally to a Hereford cow and, at intervals thereafter, samples of blood, milk, urine, and feces were assayed radiometrically and/or chromatographically.

Materials and Methods

Experimental Animal. The animal employed in this study was a lactating Hereford cow. At the time of administration of the labeled compound the cow weighed 267.6 kg.

Radioactive Compound. The phosphorus-32–labeled Diazinon was synthesized by Louloudes and associates (*10*). The radioactive compound was diluted with nonradioactive Diazinon (99.2% pure, from Geigy Agricultural Chemists Research Laboratory, Bayonne, N. J.) and, at the time of administration, the diluted material had an observable specific activity of 5.18×10^8 counts per minute per gram (mean of three determinations). The radiochemical purity of the phosphorus-32 Diazinon was found to be greater than 99% when assessed by several paper chromatographic methods of analyses.

Administration of Radioactive Compound. The labeled Diazinon was administered orally in a No. 11 gelatin capsule (0.5 ounce) by use of a balling gun. The dosage was 20 mg. per kg. (5.232 grams for the 267.6-kg. animal).

Sample Collection. The animal was held in a metal chute modified for tracer studies. Food and water were supplied throughout the experimental period.

Urine and feces were collected manually, with considerable care to prevent cross contamination. Near-quantitative collections were made for the 36-hour post-treatment interval. Subsequent to this period, samples were taken only at the various times indicated in the figures and text.

Blood samples, 10 ml., were taken from the jugular vein at each sampling; sodium citrate was used as the anticoagulant.

The animal was milked manually. Previous to milking, the udder was carefully washed and checked with a survey meter to avoid contamination of the sample. An attempt was made to remove all available milk at each sampling.

Following collection and radiometric analyses, formalin was added to the blood, milk, and urine as a preservative and the samples were held under refrigeration until extractions and/or chromatographic analyses could be performed.

Radiometric Measurements and Sample Preparation. Radiometric analyses were made with a conventional scaler and a windowless gas-flow proportional counter. All samples were prepared in duplicate, and the mean corrected counting rate was used in computing the radioactivity. The samples were counted for a sufficient time to attain a maximum standard error of $\pm 5\%$ (*2*). The counting rates were corrected for decay and expressed as microgram- or milligram-equivalents of Diazinon.

Samples were prepared by plating the materials to be analyzed on aluminum or stainless steel planchets. To ensure even distribution of the sample, disks of tissue paper, 2 cm. in diameter, were attached to the bottom of the planchets. Care was taken to keep the sample thickness below the level of appreciable self-absorption. To prevent loss of radioactivity during drying and to avoid contamination of the windowless counter, 2 to 3 mg. of Carbowax 400 (polyethylene glycol) was added to the samples.

The blood, urine, and milk, and extracts of these fluids were assayed as plated measured aliquots, as previously described. The feces were treated in the following manner:

After thorough mixing with an electric stirrer, 2-gram samples (wet weight)